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Prevalence of Methicillin Resistant
Staphylococcus aureus on computer mice on the
campus of Southern Adventist University

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In fulfillment of Southern Scholars senior project under the supervision of Dr. Ann Foster

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Abstract

Microorganisms are present everywhere in the environment. Unbeknownst to most people *Staphylococcus aureus* is a primary pathogen responsible for many skin infections and is carried by 25% of humans in the anterior nares. Due to the infective nature of *Staphylococcus aureus*, it is important to determine its prevalence on fomites by testing commonly used surfaces. For these reasons a research experiment was designed to determine the prevalence of *S. aureus* and Methicillin Resistant *S. aureus* (MRSA) in computer labs on the campus of Southern Adventist University during the fall semester of 2010. The research experiment was conducted in computer labs found in Southern's classrooms with high student usage. Moist sterile cotton applicators were used to swab computer mice, and all locations were tested six times over a period of three months. A total of 476 fomites were tested for the presence of *Staph. aureus* and MRSA, both of which were found on these surfaces. Out of the 476 fomites tested, 6% contained *S. aureus*, and 2% contained MRSA. Based on the results of the six trials performed it can be concluded that both *Staph. aureus* and MRSA are present but not widespread on the campus of Southern Adventist University. Further research should be conducted to detect change in frequency of MRSA on these surfaces.

Introduction

Microorganisms are present everywhere in the environment; most of which the population is not even aware of. Due to the infective nature of *Staphylococcus aureus*, it is important to determine its prevalence in the community in order to raise awareness. In the *Staphylococcus* genus there are thirty three different species. All of those species are known for being gram positive cocci that arrange in grapelike clusters. Most of these are nonpathogenic and are commonly found in the nasal passages of carriers. Some types have been found to exist in the bloodstream, though they prove to be no different than those found in the nasopharynx region (Eiff et al, 2001).

Since *Staphylococcus aureus* is the most recognized pathogen of this species it was chosen to be the test subject. It has been roughly estimated that about 25% of the world's population are asymptomatic carriers for this microorganism (Dyer, 2008). In general when people do succumb to a *Staphylococcus* infection they are usually skin related, such as boils or furuncles. In the extreme cases a *Staph. aureus* infection can cause pneumonia, meningitis, or bloodstream infections. Of the blood related infections, toxic shock syndrome (TSS) is one of the most dangerous and is characterized by fevers, rash formations, and hypotension; that can ultimately lead to multiple organ failure (McCormick et al, 2001). When dealing with a patient who has developed a bacterial infection the most common treatment are antibiotics, such as penicillin and methicillin.

A problem arises when the bacteria treated develops resistance to the drugs used to combat it. A prime example of this is methicillin resistant *Staphylococcus aureus* (MRSA). The prevalence of this specific strain of *Staph. aureus* is rising worldwide and it is known to cause problems in communities especially in hospital settings (Grundman

2006). A survey study done of MRSA in European hospitals from 1999-2002 found that “MRSA was highest among patients admitted to intensive care units (35%)” (Edine et al, 2004). What makes this bacterium difficult to treat is not only its resistance to methicillin but also to oxacillin, penicillin, and amoxicillin; all of which are examples of Beta-lactam antibiotics (2008). Beta lactam antibiotics work by inhibiting the synthesis of the bacterial cell wall which proves to be lethal especially on Gram positive bacteria. Microorganisms can become resistant to these types of antibiotics by expressing the enzyme beta lactamase (Herzberg 1987).

The first known epidemiological research of MRSA was done in Zurich, Switzerland (Kayser 1972). Since that date the incidence of MRSA infections has risen drastically. The extensive use of antimicrobial agents is often followed with rising numbers of resistant bacteria (Finland 1970). Currently there has been an alarming rise of both hospital-acquired infections and community-associated infections (Kuint 2007). Buckingham (2004) states, “Community-associated MRSA has emerged as a potentially invasive pathogen among children in the greater Memphis area” (Buckingham, et al., 2004). For these reasons a research experiment was set up to determine the prevalence of *Staph. aureus* and MRSA on the campus of Southern Adventist University. Computer mice were the objects tested because they are used by most computer operators and can be contaminated with bacteria. The computer mice are considered fomites because they are inanimate objects that are capable of carrying infectious organisms. The following discussion will present data collected in 2010.

Materials and Methods

Location and Swabbing Techniques

The research was performed on the campus of Southern Adventist University, located in Collegedale, Tennessee. This is a school with approximately 2,750 students. Select buildings with high student usage along the promenade were used. The computer labs in Hickman Science Center, McKee Library, Lynn Wood Hall, and Brock Hall were the test sites. Each of the above labs had the following number of computers: 25, 48, 34, and 45 respectively. The procedure consisted of using sterile cotton applicators to swab the left click button on computer mice, approximately a 3 cm² area. All locations were tested six times over a period of four months. Fifty percent of the total computers of each lab were selected at random and tested during each trial run.

Media and Incubation

The research conducted used the basic tools found in a microbiology lab, such as a Bunsen burner and an inoculation loop. Other items used include sterile cotton swabs, Mannitol Salts Agar, Blood agar, DNase agar, and Tryptic Soy Agar slants. The previous materials were all purchased from the Carolina Biological Supply Company located in Burlington, North Carolina. The following items were also used but were acquired from the Fisher Scientific Company located in Atlanta, Georgia: Staphyloslide test, Catalase test, and CHROMagar. All inoculated plates were incubated under atmospheric conditions at 37°C.

The first step in the experiment was to randomly select 50% of the computers in

the lab and tag them with a yellow sticker on the side of the tower. During each successive trial, only the computers tagged with the yellow stickers were used. The bacteria were collected from these fomites by using the sterile swabs and rotating the head while moving back and forth along the left mouse click button. These swabs were then used to inoculate prepared Mannitol Salts Agar plates. The *Staphylococci* species were isolated on Mannitol Salts Agar and incubated for 48 hours. After the allotted time these plates were taken out of the incubator to determine if a positive result for *Staph. aureus* was obtained. If fermentation had occurred, a bacterial colony was selected using an inoculation loop and re-plated onto another MSA plate, but this time for isolation purposes.

After 48 hours if a pure culture was obtained then a sample from that plate was used to perform a Gram stain (See protocol in Appendix A). It is important to note that the Gram stains were performed around 48 hours after the initial incubation period. If the selected colony was Gram positive cocci in clusters, then a new sample from the pure colony on MSA plate was used to determine if it was *Staphylococcus aureus* by performing both the Staphyloslide and Catalase test. If the bacterium selected proved to be positive for both these tests, they were plated on Blood Agar and DNase plates. The incubation period for this step was 24 hours in an incubator set at 37°C. For this step it was crucial that the plates be read after 24 hours because any period longer than that could result in a false positive.

Finally, if all of the above tests were positive then the final selective media used was CHROMagar, which allowed Methicillin Resistant *S. aureus* bacterium to be isolated. As in blood agar, these plates had to be read within 24 hours. All the samples

that came out positive on CHROMagar were then stored on Tryptic Soy Agar slants for future reference. A flow chart of the above procedure is included in Appendix B.

Results

The research was successful in obtaining numerical data on the prevalence of *Staph. aureus* and MRSA on the campus of Southern Adventist University. A compilation of all trials and total values are included in Appendix C and represented in Figure 1. Each of the above methods described were used to select for and identify MRSA. If all six trials are put together a total of 476 fomites were tested. Based on the results the building which had the highest rate of identified MRSA was the McKee Library (Figure 2). A graphic representation is included showing the number of *Staph. aureus* colonies collect based on the four months this research was performed (Figure 3). Finally Figure 4 demonstrates the prevalence of *Staph. aureus* before and after the introduction of available hand sanitizer.

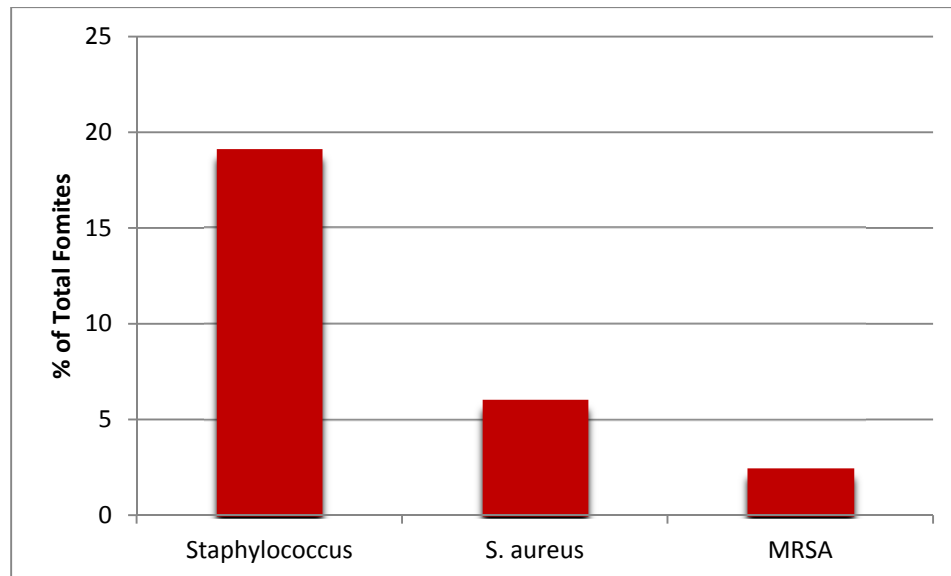


Figure 1 Ratio of *Staphylococcus* bacterium compared to the total number of samples collected. It is important to note that *Staphylococcus* group represents all Gram + cocci that were negative for the Coagulase test but positive on MSA. All of the above percentages are out of the total of 476 fomites swabbed in the fall semester of 2010.

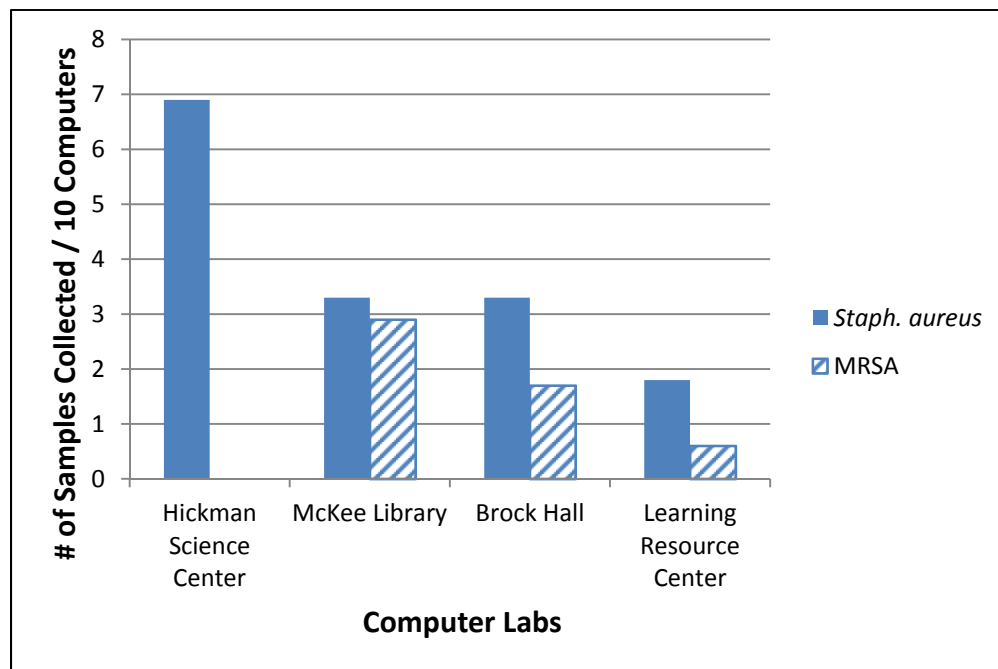


Figure 2 Prevalence of both *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* on the computer labs tested at Southern Adventist University. The values on the y-axis are correspondent to actual isolated bacteria scaled by the number of computers swabbed in each lab. As can be seen the McKee Library had the highest incidence of MRSA collected, while the Hickman Science Center had the highest incidence of *Staph. aureus*.

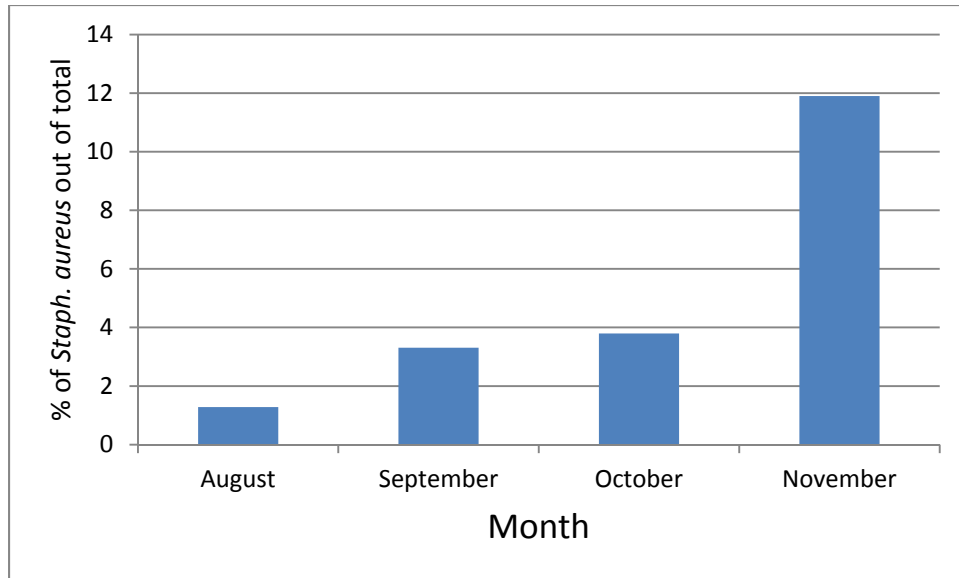


Figure 3 Relationship between the number of *Staphylococcus aureus* colonies and the month they were collected. All values represent a percent out of total fomites swabbed that were positive for *Staph. aureus* in each month. November was the month with the highest count of *Staph. aureus* isolated.

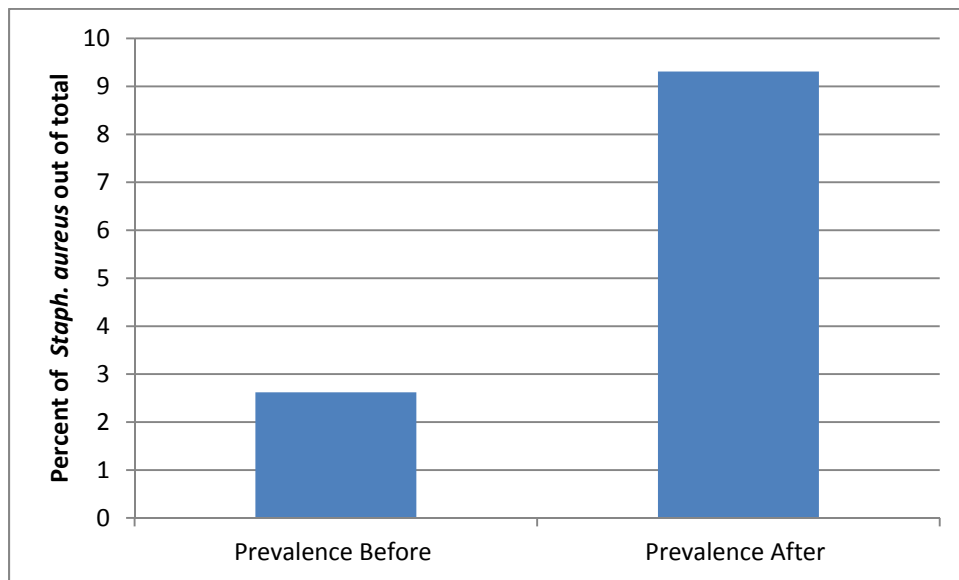


Figure 4 Comparison between the prevalence of *Staphylococcus aureus* collected before and after accessible hand sanitizer was introduced. The data from trials one to three make up the values for the before column and the data from trials four through six make up the values for the after column. Each group represents the percent of positively identified *Staph aureus* out of the total fomites swabbed for each group.

According to the above data, the overall prevalence of MRSA on campus was 2.52% of the total fomites tested, in other words twelve plates came out to be positive for MRSA. In figure 3 there seems to be a drastic increase of *Staphylococcus aureus* between the months of October and November. A possible explanation for this result could be that during the month of October the specimens were collected the day after midterm break. Therefore, the number of students using the labs would be lower. These results also suggest that *Staphylococci* bacteria are not capable of surviving for a prolonged period of time on computer mice.

Discussion

When dealing with microorganisms, selecting the correct media is important. Diverse growth factors are found on different agars and as a result they select for different bacteria. From the swabbed fomites, *Staph. aureus* and MRSA were selected for using a modified Baird Parker approach (Baird-Parker 1965). The multi-step process was designed to identify possible *Staph. aureus* candidates by beginning with the use of MSA. After a potential candidate was isolated then the second round of tests were done to see if it was truly *Staph. aureus*. Only after a sample was positively identified to be *Staph aureus*, was it plated onto CHROMagar to detect if it was MRSA. In the following paragraphs the importance of each step will be delineated.

The first selective media inoculated was Mannitol Salts Agar (MSA). This type of media is selective for *Staphylococci* species in general. It works by testing for the fermentation of the sugar, mannitol. MSA encourages the growth of Gram + *Staphylococci* while inhibiting the growth of other Gram – bacteria. In theory *Staph. aureus* results in bacterial growth along with a positive media color change from red to

yellow, while *Staph. epidermis* will grow on the media but not ferment the mannitol.

The second media used was blood agar, specifically a combination of TSA and 5% sheep's blood. The importance of this media was to test for hemolysis, the lysing of red blood cells (Mulligan et al, 1995). There are three main types of hemolysis - partial hemolysis, complete hemolysis, and no hemolysis. These are represented by Greek letters, alpha (α), beta (β), and gamma (γ), respectively. *Staphylococcus aureus* exhibits β hemolysis. It seems that increased hemolytic activity would contribute to pathogenicity.

The third media used was DNase, which is a type of specialized media that tests for the enzyme that cleaves phosphodiester linkages. These specific linkages are found on strands of either DNA or RNA. If they are broken, then the sequences are destroyed thus precipitating cell death. The agar itself is blue, so a positive test results in a halo around the inoculation area. It is important to note that the inoculation of DNase is only on a dime-shaped region in the middle of the plate and that this test does not always give accurate results. The purpose of this test is to assure a more accurate identification of *Staphylococcus aureus*. The final media used was CHROMagar, which is selective by allowing only bacteria that are resistant to antibiotics grow on it, specifically MRSA (2005, Hedin). A positive result is indicated by colored growth on the plate.

The Coagulase and Catalase tests were used because they aid to differentiate between *Staphylococci* species. The coagulase test checks for the production of the enzyme coagulase. This enzyme is responsible for converting fibrinogen into fibrin. *Staph. aureus* is coagulase positive, which means that upon performing the test one notices very distinguishable agglutination among the positive bacterial colonies (2004, Soloaga). Coagulase is believed to be found on the surface of bacteria which allow it to

exhibit clumping when it comes into contact with fibrinogen. Research has shown that this is one of the best tools available to accurately tell whether a bacterium is *Staph. aureus* or not. The Gram staining procedure was used because it is an empirical method used to classify bacteria into two major groups, Gram positive and Gram negative. This classification is based on the different bacterial cell wall properties. Also Gram staining is useful to help determine the shape and arrangement of the bacterium.

Based on the results of the six trials performed it can be concluded that both *Staphylococcus aureus* and MRSA are present at low concentrations on the campus of Southern Adventist University. It is interesting to note from figure 2, the notable absence of MRSA in the Hickman Science Center lab. Especially in light of it having the highest rate of *Staph. aureus* present. These results demonstrate that an abundance of *Staphylococcus aureus* does not necessarily correspond with an abundance of MRSA. It is also interesting to note how as the season changed from warmer to cooler temperatures the prevalence of *Staphylococcus* in general increased (Figure 3). A probable cause for the observed trend could be the fact that as temperature cools, students and staff get more prone to diseases and therefore become more active carriers. In no trial was there a prevalence of *Staph.* higher than 42% of total and the occurrence of MRSA was never higher than 5% of total. These facts alone are witness to the effort the custodial department makes to ensure that public usage areas are clean and disinfected. This research is important because it not only serves as a springboard for further research but also because it demonstrates that microorganisms such as MRSA are capable of surviving on hard contact surfaces not just moist warm environments.

One of the goals of this study was to see if introducing accessible hand sanitizer

provides for a difference in prevalence. To achieve this goal, signs were put up in noticeable areas along with a 2 Liter bottle of Member Mark hand sanitizer at each computer lab. These bottles were put into place a week before the last three trial runs. Unfortunately based on the results this antiseptic method did not result in decreased prevalence. As can be seen from the trial results in figure 4, the use of hand sanitizer did not limit the number of bacterial specimens collected. Two possible explanations for this could be that the hand sanitizer was not used by the students or that the custodial methods for the labs are effective at reducing microbial load on the computer mouse buttons.

The research conducted yielded valuable information on the prevalence of *Staphylococcus aureus* and MRSA on the campus of Southern Adventist University. For future research an increase in the number of samples could result in more consistent data. One would expect a higher prevalence rate of both *Staph. aureus* and MRSA in hospital and nursing home settings; the reason being that in small densely populated areas the probability of spreading diseases from one patient to another is high. *Staph. aureus* is pathogenic and can cause severe illnesses if not treated early. One of the best ways to prevent MRSA is to cover an infected wound and to wash hands regularly. It is important to note that the study was conducted swabbing fomites not people. It is essential to continue this research, in order to gain more data and monitor the prevalence of MRSA along with other infectious microorganisms. This research serves as a springboard to raise further questions. Additional research studies could include: differentiating the MRSA collected as being either community or hospital acquired strains, determining how long the viability is of Staphylococci bacterium on hard cold fomites, such as computer mice and quantifying if the number of different computer users impact the incidence rate.

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http://www.cdc.gov/ncidod/dhqp/ar_MRSA_ca_public.html#1

Appendix A

Gram Staining Procedure

1. Make a smear of the bacterium desired and label the slide.
2. Dry the smear.
3. Fix the smear in the Bunsen burner flame.
4. Place the slide on the rack in the staining tray.
5. Flood the smear with **Crystal Violet** solution. Allow to stand for **one minute**.
6. **Rinse** off the crystal violet carefully with water.
7. Cover the smear with **Gram's Iodine**. Allow it to stand for **one minute**.
8. **Rinse** the iodine off carefully with tap water.
9. **Decolorize** the smear with **Gram alcohol** until the blue color stops leaching out from the smear. This usually requires **10 to 15 seconds** maximum unless the smear is very thick. Do not over decolorize.
10. **Rinse** the smear with tap water.
11. Counter stain with **Safranin** for about **20 seconds**.
12. **Rinse** the slide with tap water and gently blot dry.
13. Observe under oil immersion

Appendix B

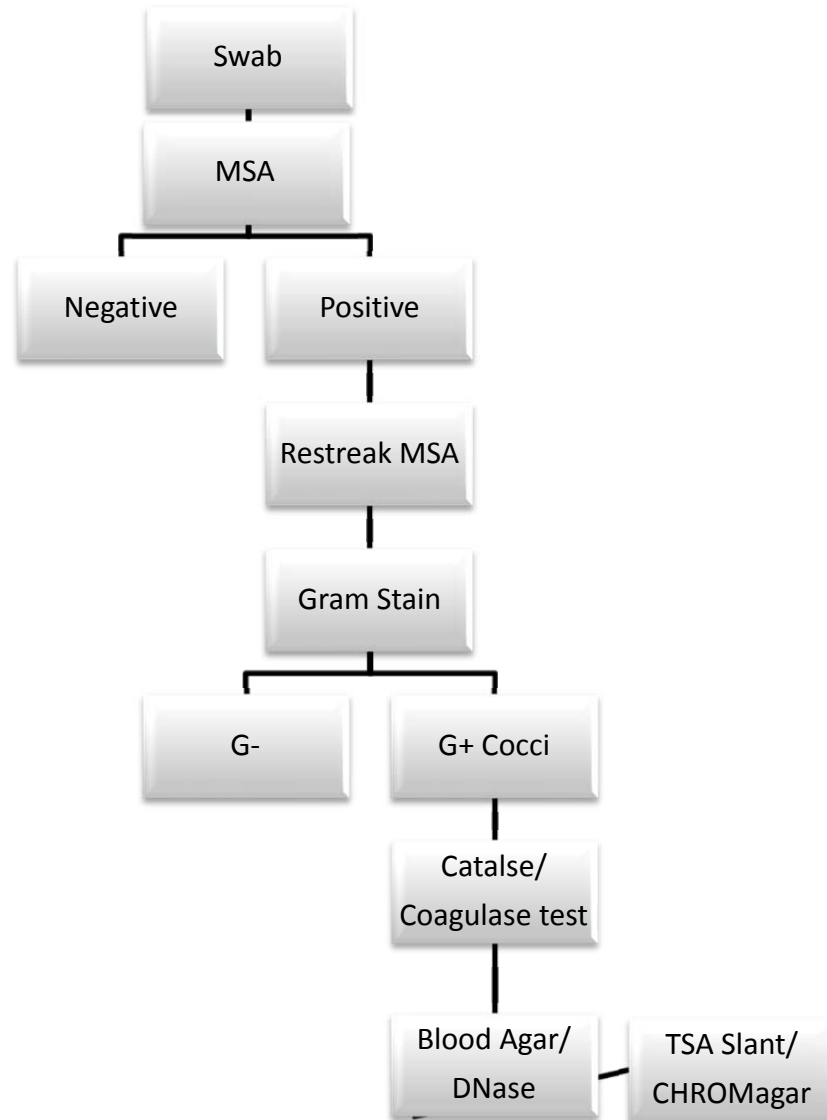


Figure 5A schematic representation of the process followed in order to positively identify both *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus*. Mannitol Salts Agar (MSA) is a growth medium that selects for Gram + bacteria that are able to ferment mannitol. A positive result is identified by a color change of the media from red to yellow. The Gram staining procedure allows for the classification of bacteria based on cell wall properties, shape, and arrangement. The catalase and coagulase test aid in differentiation between *Staphylococci* species. The coagulase test checks for the production of the enzyme coagulase. A positive result is seen when noticeable agglutination among the bacterium occur. Only *Staph. aureus* is coagulase positive, therefore it serves as the main tool to distinguish between *Staph. aureus* and *Staph. epidermis*. Blood Agar is a growth medium used to differentiate bacteria according to hemolytic patterns. *Staphylococcus aureus* exhibits complete hemolysis, while other *Staphylococci* bacteria do not. DNase is a growth medium that selects for the enzyme that cleaves phosphodiester linkages. A positive result is a rose colored halo around inoculation area. *Staph. aureus* is considered to contain the enzyme deoxyribonuclease (DNase) thus always exhibits a positive result. Finally CHROMagar is a growth medium that selects for bacteria that are resistant to antibiotics. A positive result is indicated by colored

growth on the site of inoculation. This final media is the decisive step to differentiate between *Staph. aureus* and MRSA.

Appendix C

<i>1st Trial (78 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	10.26	89.74
% <i>Staph. aureus</i>	1.28	98.72
% MRSA	0	100

Table 1 Data results in percentages for Trial 1, which was performed on August 27, 2010.

<i>2nd Trial (79 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	11.39	88.61
% <i>Staph. aureus</i>	2.53	97.47
% MRSA	2.53	97.47

Table 2 Data results in percentages for Trial 2, which was performed on September 15, 2010.

<i>3rd Trial (72 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	8.33	91.67
% <i>Staph. aureus</i>	4.17	95.83
% MRSA	4.17	95.83

Table 3 Data results in percentages for Trial 3 which was performed on September 27, 2010.

<i>4th Trial (79 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	10.13	89.87
% <i>Staph. aureus</i>	3.80	96.20
% MRSA	2.53	97.47

Table 4 Data results in percentages for Trial 4, which was performed on October 25, 2010.

<i>5th Trial (80 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	41.25	58.75
% <i>Staph. aureus</i>	8.75	91.25
% MRSA	2.5	97.5

Table 5 Data results in percentages for Trial 5, which was performed on November 8, 2010.

<i>6th Trial (88 Total)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	30.68	69.32
% <i>Staph. aureus</i>	14.77	85.23
% MRSA	2.27	97.72

Table 6 Data results in percentages for Trial 6, which was performed on November 29, 2010.

<i>Total (476)</i>		
	Positive	Negative
% <i>Staphylococcus</i>	19.12	80.88
% <i>Staph. aureus</i>	6.09	93.91
% MRSA	2.52	97.48

Table 7 Data results in percentages for all trials combined